Comparative Analysis of Cervical Cancer in Women and in a Human Papillomavirus-Transgenic Mouse Model: Identification of Minichromosome Maintenance Protein 7 as an Informative Biomarker for Human Cervical Cancer

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ABSTRACT

Human papillomaviruses (HPVs), such as HPV-16, are associated with >99% of cervical cancers in women. Two viral oncogenes, E6 and E7, are selectively expressed in these cancers. K14E6 and K14E7 transgenic mouse strains, which express the HPV16 E6 or E7 gene in stratified squamous epithelia, display many acute and long-term phenotypes indicative of the oncogenic potential of E6 and E7 including epithelial hyperplasia, abrogation of normal DNA damage responses, and spontaneous skin tumors. When treated with estrogen, these HPV-16 transgenic mice develop a progressive disease leading to cervical cancer that shows many histopathological parallels to cervical cancer in women. In this study, we evaluated the cervical lesions that arise in these transgenic mice for the expression of biomarkers induced in human cervical cancer. These analyses, which showed close parallels in the timing and pattern of expression of cyclin E and Ki-67 in the mouse cervical disease compared with that in humans, provided further validation of this HPV-16 transgenic mouse model for human cervical cancer. We then used our mouse model to identify minichromosome maintenance protein 7 (MCM7), an E2F-induced cellular DNA replication factor, as a novel biomarker for cervical cancer. In both the mouse and human disease, strong, full thickness staining for MCM7 was seen selectively in the epithelium of high-grade intraepithelial lesions and in frank cancer. The uniform staining pattern and strong signal for MCM7 suggest that MCM7 may be a highly informative biomarker for cervical cancer.

INTRODUCTION

A subset of HPVs 1 is associated with at least 99% of cervical cancers, a leading cause of cancer deaths in women worldwide (1). A common feature among cervical cancers is the selective up-regulation of two HPV genes, E6 and E7, which encode multifunctional proteins best known for their ability to inactivate the cellular tumor suppressors, p53 and pRb, respectively (reviewed in Ref. 2). Tissue culture and animal model studies have clearly established the oncogenic potential of E6 and E7. They contribute to cell immortalization in tissue culture (3), induce epithelial cell hyperplasia in vivo (4, 5), abrogate DNA damage responses (6, 7), and induce genomic instability (8, 9). Using transgenic mice expressing either E6 or E7 from HPV-16, the papillomavirus genotype most commonly associated with human cervical cancer, behind the human keratin 14 promoter, we have previously dissected the contributions of E6 and E7 in cancer both in the skin (4, 5, 10) and the cervix (11). In the cervix, E7 was found to cooperate with estrogen to induce high grade dysplasia (HSIL), carcinoma in situ, and microinvasive cancers (11). Although E6, in the absence of E7, had little effect in the cervix beyond that seen with estrogen treatment of nontransgenic mice [i.e., hyperplasia and low-grade dysplasia (LSIL)] only, the two viral oncogenes together gave rise to large, frank cervical cancers in ~25% of the estrogen-treated doubly transgenic mice, indicating a role of E6 in malignant progression (11). The histopathology of the progressive disease that arises in these HPV transgenic mice mimics closely that seen in women with cervical dysplasia and invasive carcinoma (11). In the current study, we assess the expression pattern of known biomarkers for human cervical cancer in the cervix of HPV transgenic mice and, using this animal model, identify a novel biomarker for human cervical cancer.

Ki-67 is a general proliferation marker used widely to characterize malignant lesions, including those of the cervix. The degree of Ki-67 positivity is correlated to the severity of cervical lesions: the number of cells staining positive increases with an increasing degree of dysplasia, as does the penetration of the staining into the upper layers of the epithelium (12). Cyclin E is another reported cervical cancer marker. Cyclin E stains both cervical dysplasia and cervical squamous carcinoma, and its presence is strongly associated with HPV presence (13–15). Tissue culture studies suggest that E7 is responsible for the increase in cyclin E expression and that this increase is specific to high-risk rather than low-risk E7, likely because of the heightened ability to inactivate pRb by high-risk E7 (16, 17). We examined the expression of both of these markers in our mouse model and found similarities in their timing and expression in comparison to human cervical cancer. These studies provide further validation of this HPV-16 transgenic mouse model for human cervical cancer.

We then used this mouse model to examine new potential candidate biomarkers for human cervical cancer. Because our prior studies using the HPV transgenic mice indicated E7 to contribute to the progressive cervical disease leading up to the development of cancer, we focused our search for informative markers of cellular proteins likely to be affected in their expression by E7. E7, by inactivating pRb and the related proteins p107 and p130, activates the E2F family of transcription factors and thereby induces expression of E2F-responsive genes. MCM7, a component of a cellular DNA helicase (reviewed in Ref. 18), is an E2F-responsive cellular gene (19, 20). We found MCM7 expression to be induced during the course of the progressive disease that arises in the HPV transgenic mice. MCM7 staining was restricted to basal and immediate parabasal layers in both normal and hyperplastic tissue. Full thickness staining was observed in high-grade dysplasia and invasive cancers. We found a similar pattern of induction in MCM7 expression in the progressive human disease, with strong, full-thickness staining for MCM7 seen in the epithelium of HSILs as well as in frank cancer. The uniform staining pattern and strong signal for MCM7 in these human lesions suggests that MCM7 may be a highly informative biomarker for cervical cancer.
MATERIALS AND METHODS

Histological Specimens. Histological sections were obtained from the reproductive tracts of nontransgenic K14E6 (line 5737; Ref. 4), K14E7 (line 2304; Ref. 5), and K14E6/K14E7 double transgenic female mice that had or had not been treated chronically with 17β-estradiol (0.05 mg/60-day period) for 6 months. The treatment of these mice and the histopathological characterization of these tissues have been described previously (11). All mice were of the FVB/N inbred genetic background. Histological sections from archival paraffin-embedded patient biopsies with no personal identifiers were obtained from the University of Wisconsin Hospital and Clinics.

Immunohistochemistry. Paraffin-embedded sections were deparaffinized in two changes of xylene, hydrated through a graded series of ethanol:water mixes, rinsed in PBS, and quenched for endogenous peroxidase activity by treatment with 3% H2O2 in methanol for 20 min. After washing with PBS, sections were microwaved in antigen retrieval buffer (10 mM sodium citrate, pH 6.0) for 20 min and allowed to cool at room temperature for an additional 30 min. Sections were rinsed in PBS and blocked in 1.5–5% horse serum in PBS for 30 min at room temperature. Primary antibodies (Ki-67, Dako, Carpinteria, CA, 1:25; Cyclin E, Santa Cruz Biotechnology clone M-20, Santa Cruz, CA, 1:400; Mcm7, Neomarkers, Fremont, CA, 1:200; and p16, Neomarkers clone JC8, Fremont, CA, 1:200) were diluted in blocking solution according to the manufacturer’s instructions (Vector, Burlington, CA). Sections were counterstained with hematoxylin (Gill’s formulation; Vector, Burlington, CA), dehydrated through ethanol and cleared in xylene, then mounted using Cytoseal XYL reagent (VWR, West Chester, PA).

Sections from raft cultures of the immortalized human foreskin keratinocyte cell line (NIKS, also known as BC1-Ep/SL) harboring no viral DNA, wild-type HPV-16 genomes, or E7 null HPV-16 genomes (also referred to as E7TTL mutant genomes), which were generated previously (21), were stained for MCM7 as described above, and four ×20 microscopic fields from two or more independent rafts were quantified for the frequency of MCM7-positive cells. For detection of apoptosis, sections of cervical tumors were stained with the Apoptag Plus Fluorescein In Situ Detection Kit (Intergen #S7111, Purchase, NY) according to the manufacturer’s instructions, after being deparaffinized and rehydrated as described above. After extensive washes in PBS, sections were mounted in Vectashield mounting medium containing propidium iodide counterstain (Vector).

RESULTS

Expression of Established Biomarkers for Human Cervical Cancer in the Mouse Model for HPV-associated Cervical Cancer. A number of cellular factors are induced in human cervical cancers and their precursor lesions, and several are now being tested or used as biomarkers for diagnosing and/or staging cervical cancers and their precursor lesions in the clinic, with varying degrees of success. Histopathologically, the progressive disease that arises in the cervixes of HPV-16 transgenic mice (K14E6 and K14E7), when treated with estrogen, compares favorably to that seen in patients who develop HPV-associated human cervical cancer (11). To evaluate further the relevance of our mouse model for HPV-associated cervical cancer, we investigated the pattern of expression of several known biomarkers for human cervical cancer. These included Ki-67 and cyclin E.

Ki-67, a marker for proliferating cells (22) and a reported biomarker for human cervical cancer (23, 24), was restricted in its expression to the basal or parabasal compartment of the hyperproliferative stratified squamous epithelium in the cervixes of estrogen-treated, non transgenic mice (Fig. 1A). A similar pattern of staining was seen in low-grade dysplasia (LSIL) that arose in the estrogen-treated K14E6 mice (Fig. 1B). This pattern of expression was indistinguishable from that seen in untreated nontransgenic and K14E6 transgenic mice, which also showed staining limited to the basal and parabasal strata (data not shown). These results indicate that hyperplasia and LSIL, the two early stages in progressive cervical disease in our mouse model, do not lead to any abnormalities in the distribution of proliferating cells within the epithelium. In contrast, Ki-67 expression was induced in the HSIL-grade neoplastic stratified squamous epithelia of the estrogen-treated K14E7 mice with many Ki-67-positive cells observed throughout the suprabasal compartment of the tissue (Fig. 1C). In the large frank cancers of the cervix that arise in the estrogen-treated K14E6/K14E7 double transgenic mice, some Ki-67-positive cells were again found throughout the lesions (Fig. 1E). Biomarker staining could not be evaluated in low-grade lesions in either the

Fig. 1. Ki-67 expression in the mouse cervix. Shown are histological cross-sections of the mouse cervical tract from nontransgenic and HPV-transgenic mice treated with estrogen for 6 months. Sections were stained with Ki-67 monoclonal antibody and counterstained with hematoxylin. Ki-67-positive cells show brown staining in the nucleus. Note that detection of Ki-67 is restricted to the basal and parabasal layers of the cervical squamous epithelium in both the hyperplasia (A) and LSIL (B), whereas scattered staining is evident throughout multiple layers of cells in the HSIL (C). Ki-67 is also detected in a subset of cells throughout the frank cancers (E). D, a H&E stain of the tissue shown in E, included for orientation.
K14E7 or K14E6/K14E7 because of the presence of highly dysplastic epithelium throughout the entire cervical squamous epithelium. Interestingly, the Ki-67 staining was not uniform in the HSILs and cervical cancer lesions in the mouse model. This non-uniform pattern of Ki-67 staining is similar to that observed in human cervical cancer where the percentage of cells staining positively for Ki-67, reported to range from 41 to 82% in frank cancer and 41 to 87% in HSILs (23), reflects a potential limitation in the usefulness of Ki-67 as a biomarker in the clinic.

**Cyclin E**, an E2F-regulated cell cycle gene (25, 26) and another reported biomarker in human cervical cancer (14, 15, 27, 28), was not detected in the hyperplastic stratified squamous epithelia of estrogen-treated nontransgenic mice (Fig. 2A) nor in the LSILs in estrogen-treated K14E6 transgenic mice (Fig. 2B). In contrast, some cyclin E staining was seen in all portions of the HSILs in the estrogen-treated K14E7 mice (Fig. 2C) as well as in the larger frank cancers of the cervix found in the estrogen-treated, K14E6/K14E7 doubly transgenic mice (Fig. 2D). As with Ki-67, not every cell within a lesion of the treated K14E7 or K14E6/K14E7 mice stained positively for cyclin E; rather the staining was intermittent. This is similar to what has been seen in human cervical cancers and high-grade dysplastic precursor lesions (11), again reflecting a potential limitation in the usefulness of this biomarker. One difference was seen between the staining pattern for cyclin E in LSILs from our mouse model and that of human samples. In the LSILs arising in the estrogen-treated K14E6 mice, no cyclin E staining was observed. In contrast, cyclin E staining is seen in LSIL samples from women. We believe this difference reflects the absence of E7 expression in the K14E6 mice. **Cyclin E** is an E2F-responsive gene (25) that is likely deregulated in its expression by E7's inactivation of pRb and perhaps the other pocket proteins p107 and p130 (26).

On the basis of the data summarized in Figs. 1 and 2, the progressive disease in the mouse model for HPV-associated cancers shows close similarity in the pattern of expression of two established biomarkers of human cervical cancer, Ki-67 and cyclin E, to that seen in the progressive human disease. These results further validate the relevance of this mouse model for HPV-associated cervical cancer.

**Identification of a Novel Marker for Progressive Disease in the Mouse Model for HPV-associated Cervical Cancer.** A number of biomarkers for human cervical cancer have been identified, including the ones tested above (Ki-67 and cyclin E) in our mouse model as well as p16, which could not be evaluated in mice because of a lack of suitable antibodies.5 In most cases (e.g., cyclin E and Ki-67), the lack of strong uniform staining within lesions and/or inconsistencies in positive readouts for the biomarker among different cervical cancers have detracted from their utility in clinical diagnostic screening. Additionally, some markers such as p16, although quite specific and highly penetrant for HSILs and frank cancer in the stratified squamous epithelium of the human cervix (13, 29, 30), also stain positively in nearby normal glandular epithelium, potentially leading to false positives in clinical screening (31). For these reasons, we were interested in identifying additional biomarkers for cervical cancer using our mouse model.

The E7 protein is known to bind and inactivate pRb and the related p107 and 130 proteins, all of which normally regulate the activity of the E2F family of transcription factors. Therefore, E2F-responsive genes should be up-regulated in the presence of HPV-16 E7. **Cyclin E** is one such gene; however, there are many others that could be more sensitive indicators of E2F deregulation. Using a tissue culture system for studying the HPV-16 life cycle, we tested this premise. Human foreskin keratinocytes harboring wild-type HPV-16 genomes or an E7-null HPV-16 genome were grown in organotypic (raft) culture to generate stratified squamous epithelium in which the HPV-16 life cycle can be recapitulated and E7 plays a critical role (21, 32). Histological sections of these raft cultures were stained for **MCM7**, an E2F-responsive gene (19, 33) that is induced at the RNA level in

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5 Our unpublished observations and Ron DePinho and Ned Sharpless, personal communication.
HPV-positive cells based on microarray analyses. MCM7 was found to be highly induced in the raft culture harboring the wild-type HPV-16 genome (Fig. 3B) compared with the raft culture harboring no viral DNA (Fig. 3A), with the induction evident in both the basal and suprabasal compartments of the epithelium. This induction, particularly in the suprabasal compartment, was nearly completely absent in the raft culture harboring the E7-null mutant HPV-16 genome (Fig. 3C). We demonstrated previously that E7 is responsible for reprogramming suprabasal cells to re-enter the cell cycle and support DNA synthesis, allowing for the vegetative amplification of the viral genome (21). The current result demonstrates that MCM7 is induced in the natural host cell in an E7-dependent manner that correlates with E7’s ability to cause cells to re-enter the cell cycle.

Given that MCM7 is a marker for E7’s induction of DNA synthesis in the viral life cycle, we evaluated whether it also is a marker for HPV-associated cancer in the mouse cervix. In the hyperplastic cervical epithelium of estrogen-treated nontransgenic mice (Fig. 4A) and the LSILs in estrogen-treated K14E6 transgenic mice (Fig. 4B),
MCM7 staining was restricted to the basal and parabasal layers, as was seen in these mouse strains without estrogen treatment (data not shown). In the estrogen-treated K14E7 and K14E6/K14E7 mice, MCM7 staining was uniformly and strongly positive throughout the full thickness of the high-grade dysplasias (HSILs; Fig. 4C), micro-invasive cancers (Fig. 4D), as well as the large, frank cancers (Fig. 4E). This expansion in MCM7 staining correlates with the expansion in the proliferative compartment within these tissues, as reflected in the Ki-67 immunohistochemistry (Fig. 1) as well as by bromodeoxyuridine incorporation (data not shown). The MCM7 staining pattern, however, was much stronger and more uniform than that seen with either Ki-67 or cyclin E. Thus, in the mouse model, MCM7 proves to be specifically and uniformly induced in high-grade dysplasia and frank cancer of the cervix. As such, MCM7 represents a potentially valuable candidate biomarker not only for detecting frank cancer but also for distinguishing HSILs from LSILs.

**MCM7 Is a Highly Specific Biomarker for High-Grade Lesions and Frank Cancer in the Human Cervix.** To investigate the potential utility of MCM7 as a biomarker in human cervical disease, we stained archival histological sections of cervixes from patients with progressive cervical disease as well as normal tissue. The pattern of MCM7 staining in normal cervixes (Fig. 5A) and in LSILs (Fig. 5B) was similar, with positive cells limited to the basal and parabasal layers of the normal stratified squamous epithelium and expanded within the lower third of the epithelium in LSILs. In contrast, in HSILs (Fig. 5C) and in frank cancer (Fig. 5D), >90% of the squamous cells within the lesion stained positively for MCM7. The only exception to their being uniformly strong MCM7 staining in cancers were in regions of cancers with high concentrations of necrotic/apoptotic cells (Fig. 6) or inflammatory infiltrates (as judged by CD45 staining; data not shown).

To evaluate the potential value of MCM7 as a biomarker for cervical disease in humans, we evaluated the staining pattern in archival, paraffin-embedded tissue from multiple patients with normal cervical epithelium or progressive cervical disease (Table 1). We found MCM7 to be induced in all frank cancers and HSILs and un-induced in all normal and LSIL tissues. Within the HSILs, we found the expansion of MCM7 staining to correlate closely with CIN II versus CIN III subgrades of disease. The two HSILs limited to CIN II grade disease had an incomplete expansion (two-thirds of the thickness in the staining pattern for MCM7). All four HSILs with CIN III grade disease characteristics had uniform and complete expansion of MCM7 staining pattern to the full thickness of the stratified squamous epithelium, with one exception, a lesion that showed full-thickness staining in some portions and two-thirds thickness in other portions. These results indicate that MCM7 is a highly informative biomarker for human cervical cancer and the progressive disease that leads to the development of cervical cancer.

**Comparison of MCM7 Staining Patterns in Human Cervical Disease to That of p16.** p16 is currently considered an informative biomarker for human cervical cancer because it is induced in high-grade dysplasias, which are likely to give rise to cancers as well as to be contained in the cancers themselves (29, 34). Given that we likewise see a similar strong and uniform induction of MCM7 staining in the same subset of lesions, we directly compared the MCM7 and p16 staining patterns in the same human clinical samples. A very similar pattern of stain for MCM7 and p16 was found, with strong and uniform induction in the HSILs and invasive cancers with both markers (Fig. 5). The only difference was that MCM7 is restricted in its staining to the nucleus, whereas p16 staining is seen in both the nucleus and cytoplasm. Also, p16 did not uniformly stain positively the basal/parabasal cells of the normal cervix (Fig. 5E) or LSILs (Fig. 5F).

**DISCUSSION**

In this study, we evaluated the expression patterns of known biomarkers of human cervical cancers as a means of further validating our animal model for HPV-associated cervical cancer. The similarities in the Ki-67 and cyclin E staining patterns in the progressive cervical disease in our mouse model compared to that in humans verifies that our model is targeting molecules of physiological relevance to the development of cervical cancer in women. This biomarker analysis, together with the prior knowledge that the histopathology of the progressive disease arising in our mouse model closely compares with that in women (11), provides strong validation of this animal model.

In our animal model, as seen in human cancers, the pattern of induction of Ki-67 and cyclin E was not robust. Only a fraction of cells stained positively for these markers even in frank cancer. These limitations raise concern over the utility of Ki-67 and cyclin E as informative biomarkers in diagnosing cervical cancer. It is also interesting to note that our analyses of the E6 versus E7 transgenic mice allowed us to correlate induction of expression of Ki-67 and cyclin E specifically with E7 expression. This correlation, at least in the case of Ki-67, may reflect the fact that in our hands, the disease arising in the E6-only mice is limited to LSILs (CIN I). It also could reflect an enhanced ability of E7 to induce these biomarkers. Consistent with this, E7 can induce cyclin E in differentiating cultures of human keratinocytes (16). Cyclin E was likewise induced to some degree in the cervixes of some E7 transgenic mice, even in the absence of estrogen treatment (data not shown).

Using our animal model we identified MCM7 to be a novel and highly informative biomarker for human cervical cancer. Our premise for studying MCM7 arose from the analysis of the role of E7 in the HPV life cycle. We had described previously a role of E7 in reprogramming terminally differentiating stratified squamous epithelia to support DNA synthesis and specifically the vegetative amplification of the viral DNA genome (21). We have hypothesized that this ability of E7 to reprogram cells to support DNA synthesis is reflective of its inactivation of pRb, the cellular tumor suppressor that modulates the activity of the E2F family of transcription factors. We therefore predicted that E2F-responsive genes would be up-regulated by E7, and this up-regulation would correlate with the induction of DNA synthesis in the suprabasal compartment of HPV-infected epithelia when grown in organotypic culture. This proved the case as we demonstrated in Fig. 3. Indeed, the induction of MCM7 was greatest in the suprabasal compartment in which E7 reprograms cells to support DNA synthesis, the same cellular compartment in which E7's own expression is heightened during the life cycle (35). Clearly, the induction is dependent upon E7, given the absence of induction in cells harboring an E7-null genome. These data provide further support for the role of E7’s inactivation of pRb in mediating its role in the viral life cycle and provided the impetus for us to monitor MCM7 expression in cervical cancer.

In the progressive cervical disease in our mouse model, MCM7 was highly induced in HSILs and frank cancers and arose in mice only expressing E7, consistent with it being a readout for E7 function. In human cervical disease, a very similar pattern of expression of MCM7 was seen. In contrast to Ki-67 and cyclin E, MCM7 was uniformly and highly induced throughout more aggressive HSILs (CIN III) and frank cancer in the human lesions (Table 1), similar to that seen with p16. In the less aggressive HSILs (CIN II), we noted an intermediate staining pattern in which the bottom two-thirds of the cervical epithelium uniformly stained positively for MCM7, compared with the full-thickness staining in the more aggressive HSILs (CIN III). Thus, MCM7 provides one the ability to discriminate between the severity of HSIL disease. In addition, we found 100% of the human HSILs and...
frank cancers to stain robustly for MCM7, demonstrating this biomarker to be highly informative. MCM7 was also detectable without unmasking steps in CaSki cells prepared in a mock ThinPrep PAP smear sample (data not shown), indicating that surveillance for this marker is compatible with current clinical sampling for early detection of cervical disease.

The value of MCM7 in discriminating between stages of the progressive cervical disease in women compares well with other biomarkers. Interestingly, two other MCMs, MCM2 and MCM5, have also been reported by Coleman and colleagues (36, 37) to be informative biomarkers for distinguishing different stages of cervical disease in women. As with our study, MCM2 and MCM5 were highly induced in HSILs, more so than in LSILs. No description of potential differences between CIN II and CIN III lesions was reported for these MCMs. These investigators also mentioned that MCM7 labels cervical cancers (37); however, an evaluation of MCM7 staining pattern in progressive cervical disease (i.e., LSILs and HSILs) was not reported. Thus, multiple MCMs appear to be highly induced in a manner that is informative in discriminating between different stages of human cervical disease. Perhaps this is not surprising because it is now recog-
recognized that many if not all MCM genes are E2F inducible (19, 33). Whether their up-regulation is reflective of increased expression of HPV E7 that arises in progressive cervical disease, however, is not clear. High-risk HPVs are commonly found integrated in cervical cancers, leading to the selective up-regulation of expression of two viral genes, E6 and E7. These integration events, however, are not often detected in the precursor disease including HSILs. Therefore, it is reasonable to conclude that other alterations in cell physiology prior to the integration of the HPV genome may be contributing to the induction of MCM expression. Both E6 and E7 induce genomic instability (8, 9), potentially through the induction of centrosome abnormalities (38). Such centrosome abnormalities and genetic instability have been noted in the cervical epithelium of our HPV transgenic mice (11) and could contribute to changes in cell physiology that lead to the induction of MCM7.

Note Added in Proof

In the process of preparing this study for publication, we learned from Dr. John Doorbar (Cancer Research UK, London, United Kingdom) that his group performed a study (K. Middleton et al., J. Virol. 77: 10186–10201, 2003) in which they found a similar MCM7 staining pattern in human cervical cancer as seen in our study (personal communication), providing independent demonstration of the value of MCM7 as a potentially useful biomarker. This study was recently published (see Middleton et al., J. Virol. 77: 10186–10201, 2003).

ACKNOWLEDGMENTS

We acknowledge Amy Liem for assistance with mouse husbandry, estrogen treatments, and necropsies; Elsa Flores for generation of raft cultures; Anh Do for MCM7 immunohistochemistry on the raft cultures; and Harlene Edwards and Jane Weeks of the University of Wisconsin Comprehensive Cancer Center Histology Core Facility for expert histological preparation of all tissue samples analyzed. We are grateful to Josephine Harter for assistance in histopathological diagnoses.

REFERENCES

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